#4

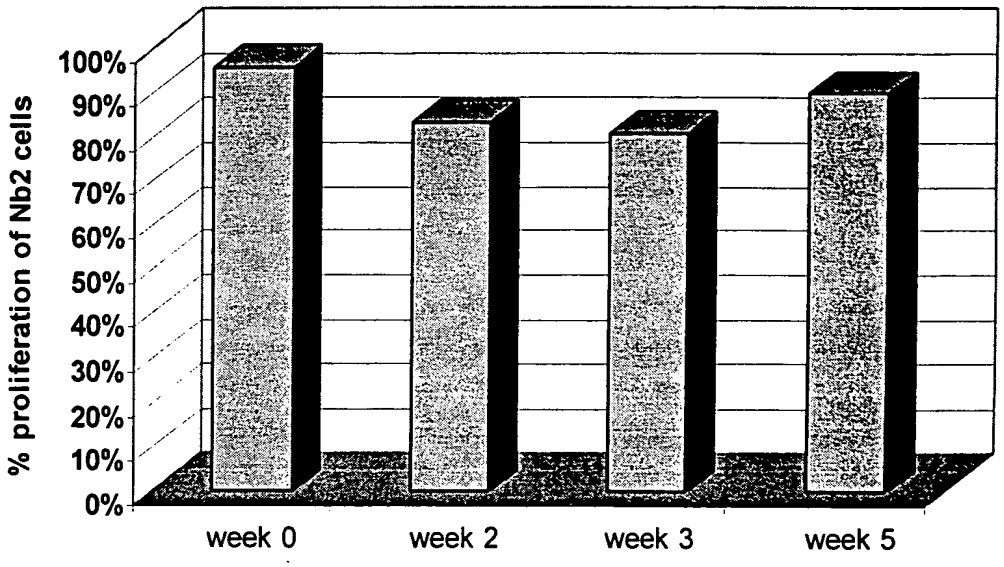

% proliferation of Nb2 cells
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
week 0
week 2
week 3
week 5

Figure 1

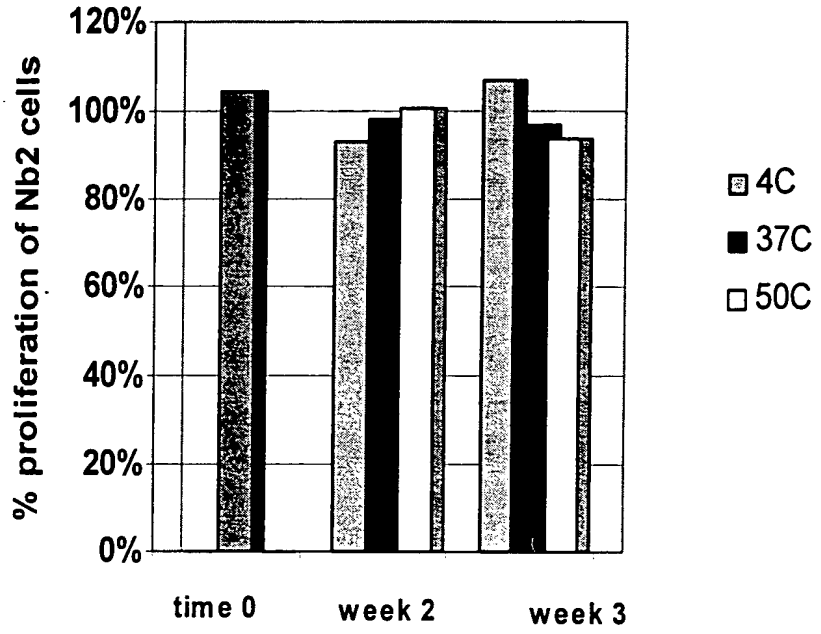

120%
100%
80%
60%
40%
20%
0%
% proliferation of Nb2 cells
time 0
week 2
week 3
4C
37C
50C

Figure 2

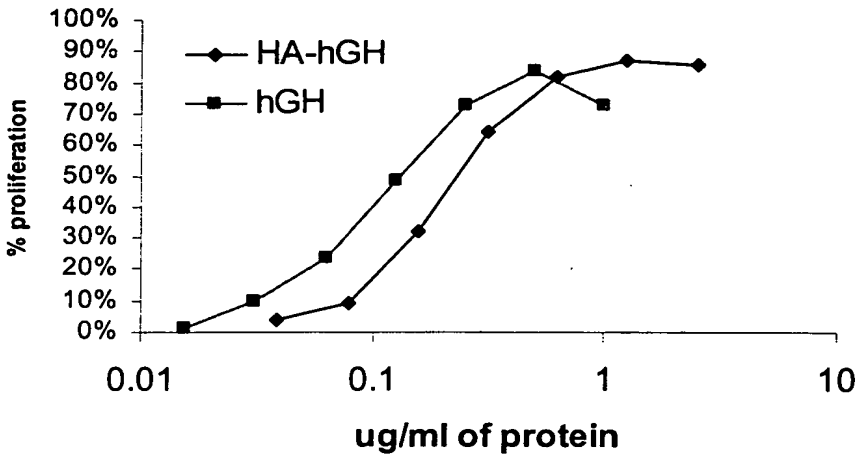


Figure 3A

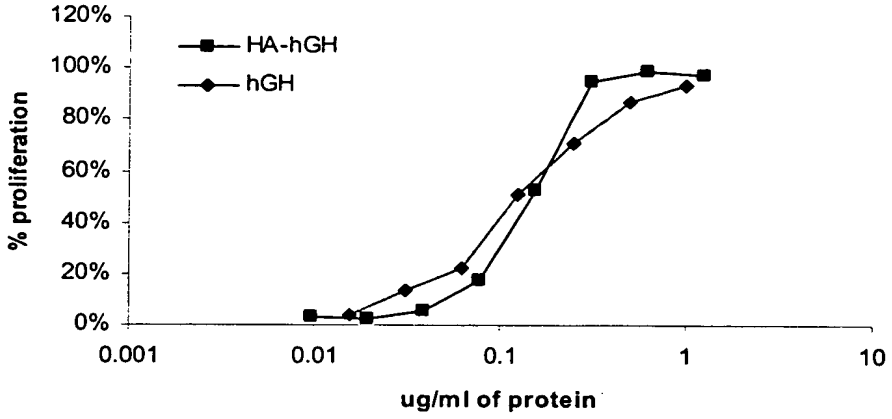


Figure 3B

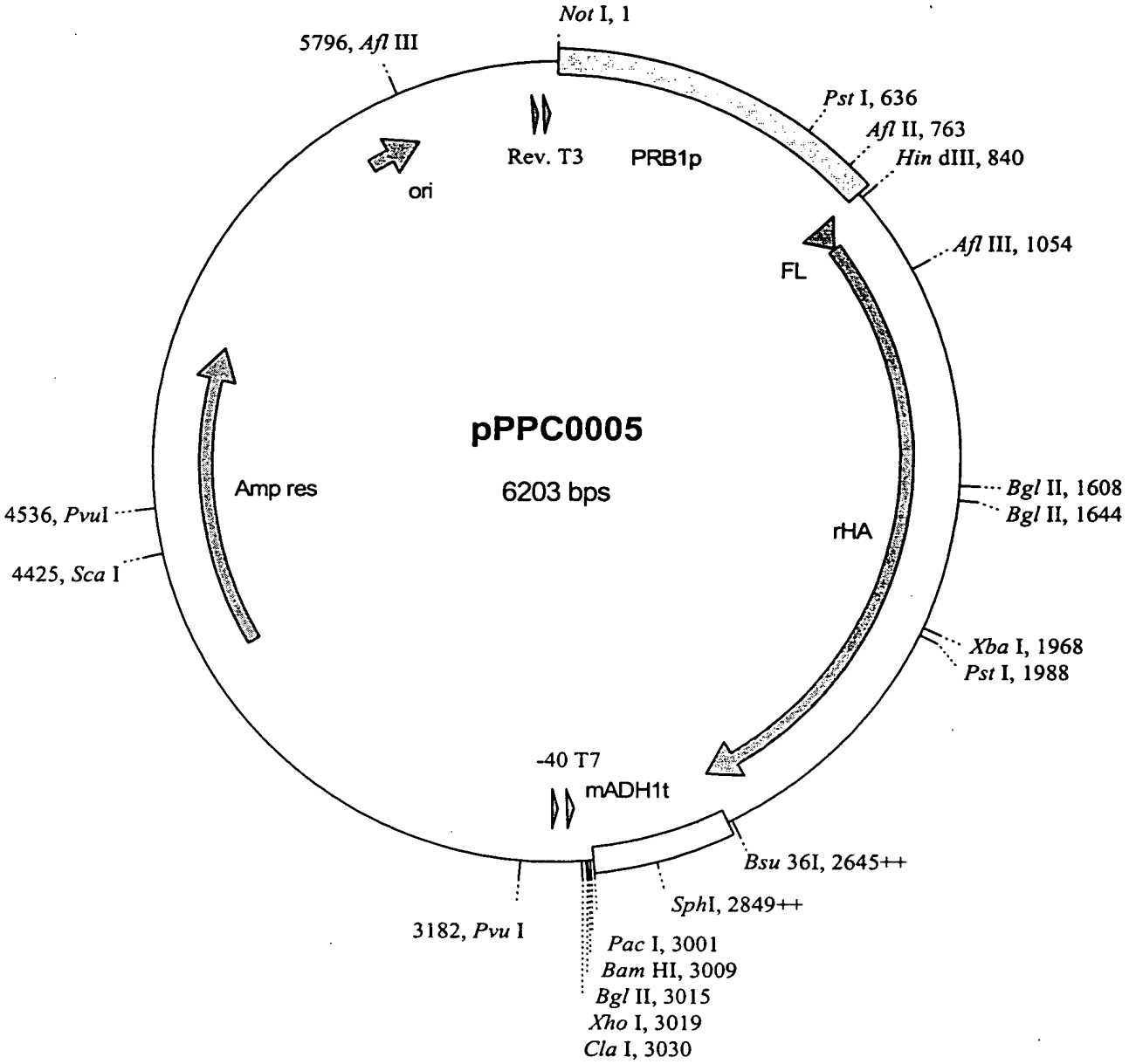

pPPC0005
6203 bps
Not I, 1
Pst I, 636
Afl II, 763
Hin dIII, 840
Afl III, 1054
Bgl II, 1608
Bgl II, 1644
Xba I, 1968
Pst I, 1988
Bsu 36I, 2645++
SphI, 2849++
Pac I, 3001
Bam HI, 3009
Bgl II, 3015
Xho I, 3019
Cla I, 3030
3182, Pvu I
4425, Sca I
4536, PvuI
5796, Afl III
Rev. T3
PRB1p
ori
FL
rHA
-40 T7
mADH1t
Amp res

Figure 4

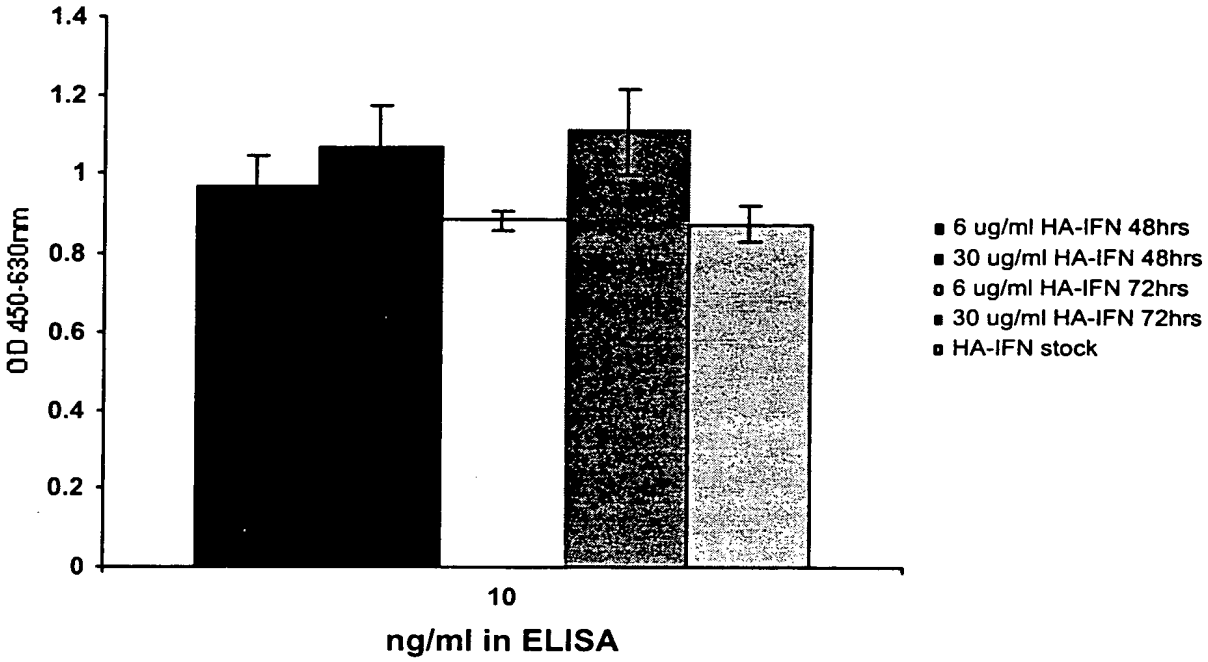

1.4
1.2
1
0.8
0.6
0.4
0.2
0
OD 450-630nm
10
ng/ml in ELISA
6 ug/ml HA-IFN 48hrs
30 ug/ml HA-IFN 48hrs
6 ug/ml HA-IFN 72hrs
30 ug/ml HA-IFN 72hrs
HA-IFN stock

Figure 5

Figure 6

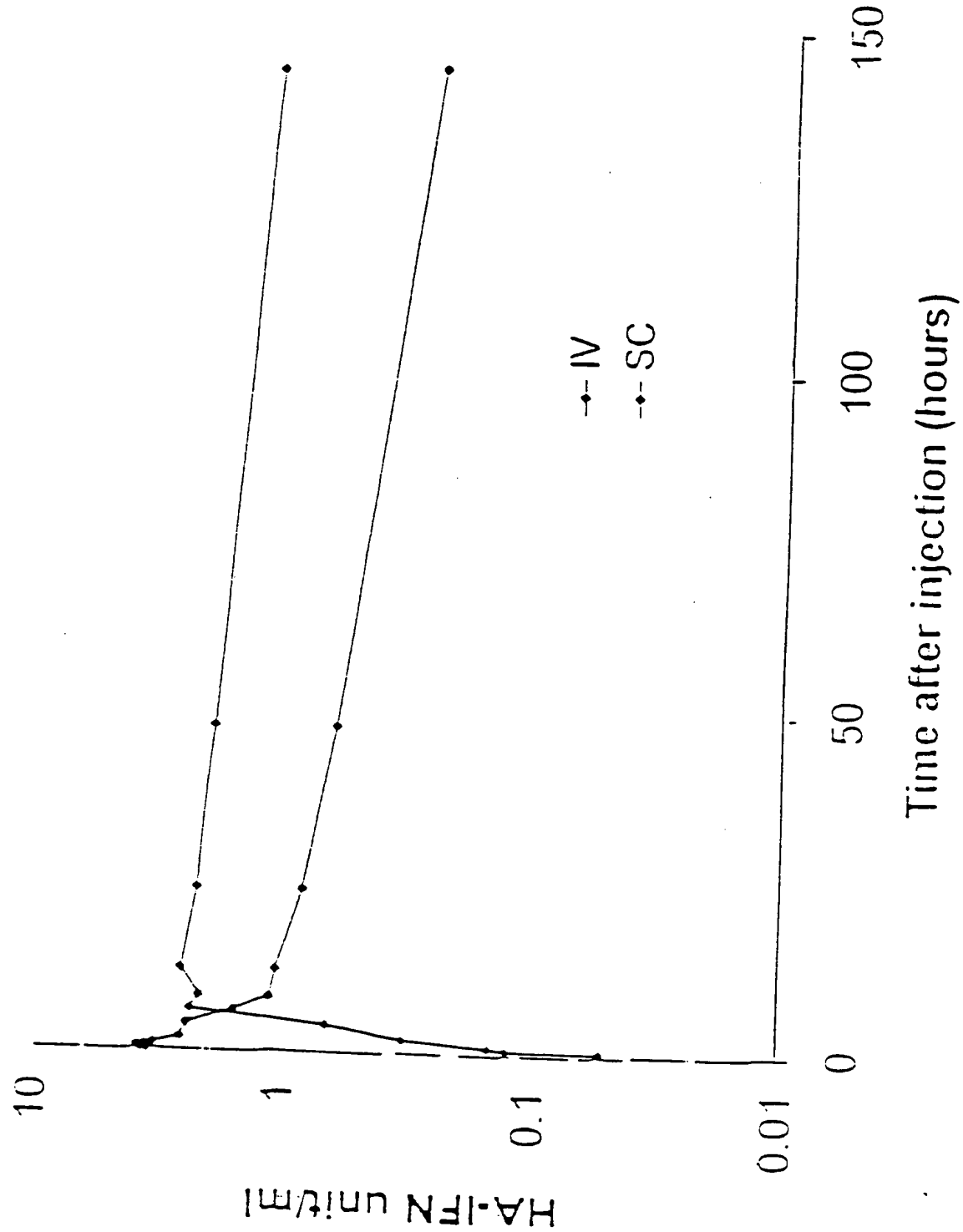
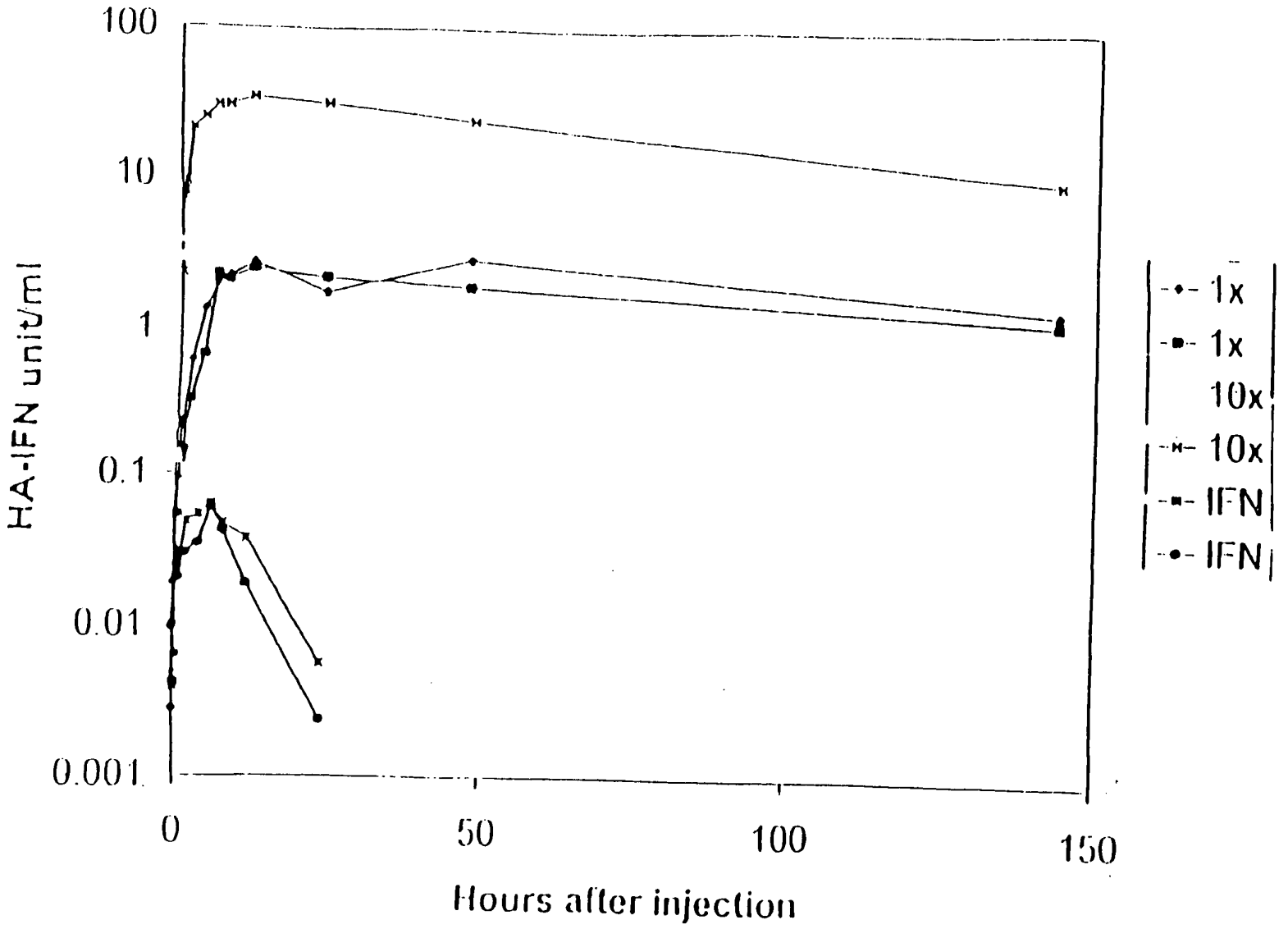


Figure 7



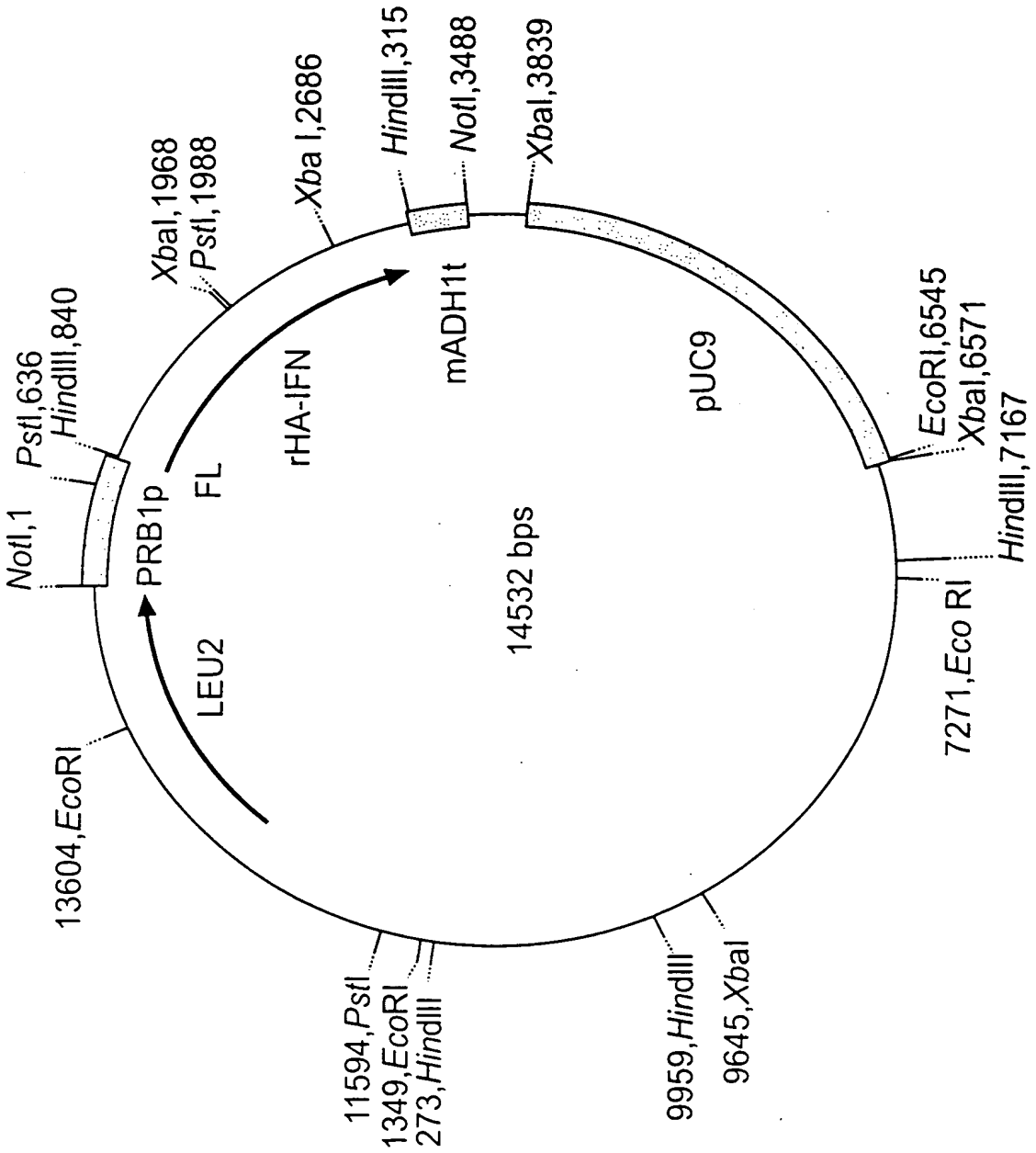

NotI,1
PstI,636
HindIII,840
XbaI,1968
PstI,1988
Xba I,2686
HindIII,315
NotI,3488
XbaI,3839
EcoRI,6545
XbaI,6571
HindIII,7167
7271,Eco RI
9645,XbaI
9959,HindIII
11594,PstI
1349,EcoRI
273,HindIII
13604,EcoRI
PRB1p
LEU2
FL
rHA-IFN
mADH1t
pUC9
14532 bps

FIG. 8

Figure 9

```
1      DAHKSEVAHR FKDLGEENFK ALVLIAFAQY LQQCPFEDHV KLVNEVTEFA
            HHHHH HHH      HHH HHHHHHHHHH      HHHHH HHHHHHHHHH

            I                             II             III
51     KTCVADESAE NCDKSLHTLF GDKLCTVATL RETYGEMADC CAKOEPERNE
       HHHHH           HHHHH HHHHH                HHHH H     HHHH

101    CFLQHKDDNP NLPRLVRPEV DVMCTAFHDN EETFLKKYLY EIARRHPYFY
       HHHH                H HHHHHHHH    HHHHHHHHH HHHHH

                                  IV
151    APELLFFAKR YKAAFTECCO AADKAACLLP KLDELRDEGK ASSAKQRLKC
       HHHHHHHHHH HHHHHHHHH        HHHHH HHHEHHHHHH HHHHHHHHHH

                                                          V
201    ASLQKFGERA FKAWAVARLS QRFPKAEFAE VSKLVTDLTK VHTECCHGDL
       HHHHH    HH HHHHHHHHHH HH        HHH HHHHHHHHHH HHHHHH   HH

                            VI                VII
251    LECADDRADL AKYICENODS ISSKLKECCE KPLLEKSHCI AEVENDEMPA
       HHHHHHHHHH HHHHH           HHHHH      HHHHHHH H

301    DLPSLAADFV ESKDVCKNYA EAKDVFLGMF LYEYARRHPD YSVVLLLRLA
            HHHH        HHHHHH     HHHHHHH HHHHHH      HHHHHHHH

                   VIII
351    KTYETTLEKC CAAADPHECY AKVFDEFKPL VEEPQNLIKQ NCELFEQLGE
       HHHHHHHHHH       HH      H    HHHHH HHHHHHHHHH HHHHHHH

                                                      IX
401    YKFQNALLVR YTKKVPQVST PTLVEVSRNL GKVGSKCCKH PEAKRMPCAE
       HHHHHHHHHH HHHH       H HHHHHHHHHH     HHH          HHHHHHHH

                       X                    XI
451    DYLSVVLNQL CVLHEKTPVS DRVTKCCTES LVNRRPPCFSA LEVDETYVPK
       HHHHHHHHHH HHHHH      HHHHHHHHH      HHHHHHHH

501    EFNAETFTFH ADICTLSEKE RQIKKQTALV ELVKHKPKAT KEQLKAVMDD
                  HHH    HHH HHHHMMEHHH HHH              HHHHHHHH

                  XII
551    FAAFVEKCCK ADDKETCFAE EGKKLVAASQ AALGL
       HHHHHHHH             HHHH HHHHHHHHHH HH
```

Loop		Loop	
I	Val54-Asn61	VII	Glu280-His288
II	Thr76-Asp89	VIII	Ala362-Glu368
III	Ala92-Glu100	IX	Lys439-Pro447
IV	Gln170-Ala176	X	Val462-Lys475
V	His247-Glu252	XI	Thr478-Pro486
VI	Glu266-Glu277	XII	Lys560-Thr566

Figure 10

a. Randomisation of Loop IV.

```
                                     IV
151   APELLFFAKR YKAAFTECCQ AADKAACLLP KLDELRDEGK ASSAKQRLKC
      HHHHHHHHHH HHHHHHHHH         HHHHH HHHHHHHHHH HHHHHHHHHH
```

```
                                     IV
151   APELLFFAKR YKAAFTECCX XXXXXXCLLP KLDELRDEGK ASSAKQRLKC
      HHHHHHHHHH HHHHHHHHH         HHHHH HHHHHHHHHH HHHHHHHHHH
```

X represents the mutation of the natural amino acid to any other amino acid. One, more or all of the amino acids can be changed in this manner. This figure indicates all the residues have been changed.

b. Insertion (or replacement) of Randomised sequence into Loop IV.

```
                                  (X)n
                                   ↓
                                     IV
151   APELLFFAKR YKAAFTECCQ AADKAACLLP KLDELRDEGK ASSAKQRLKC
      HHHHHHHHHH HHHHHHHHH         HHHHH HHHHHHHHHH HHHHHHHHHH
```

The insertion can be at any point on the loop and a length where n would typically be 6, 8, 12, 20 or 25.

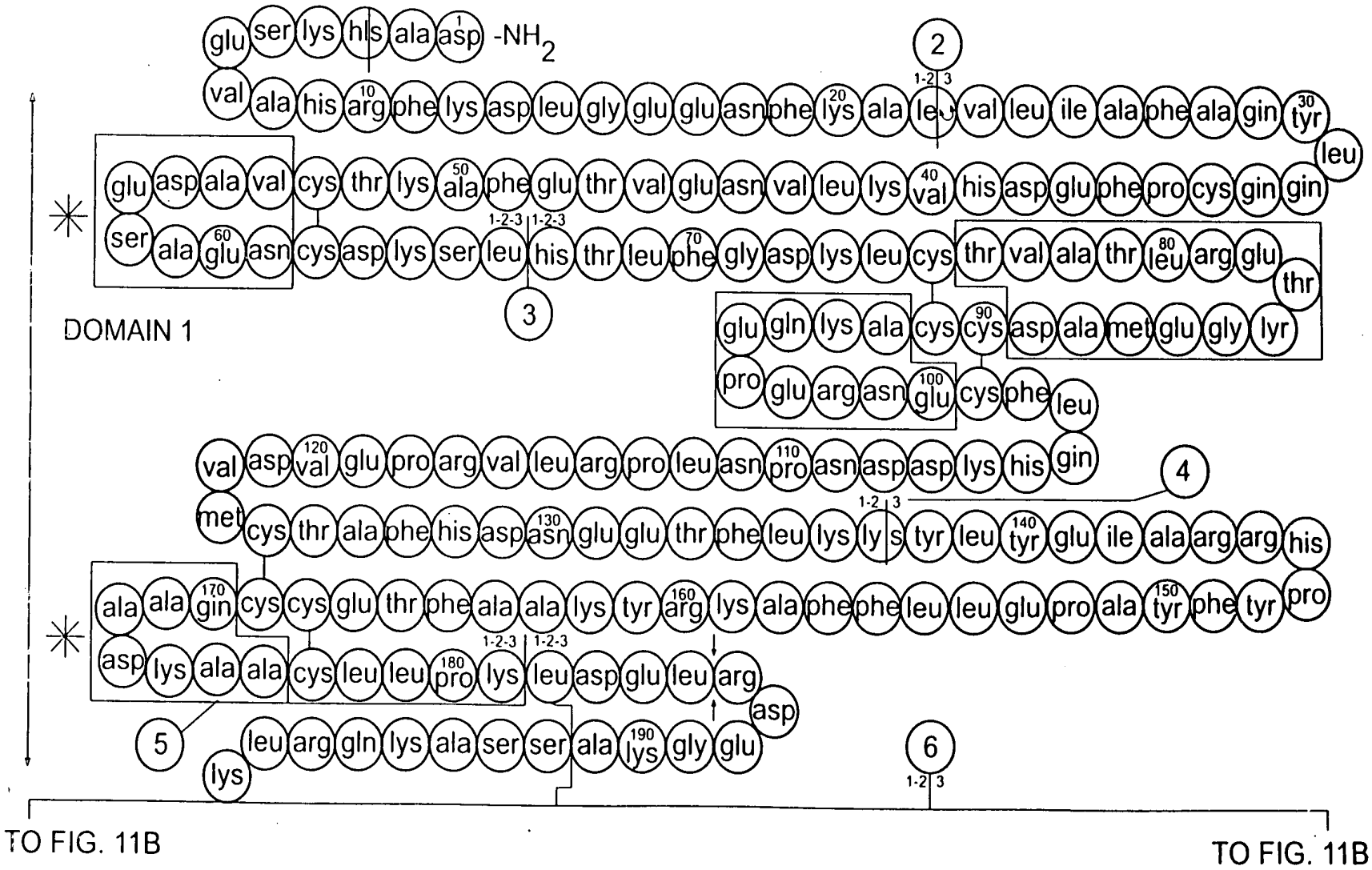

glu ser lys his ala asp -NH2
val ala his arg phe lys asp leu gly glu glu asn phe lys ala leu val leu ile ala phe ala gln tyr leu
glu asp ala val cys thr lys ala phe glu thr val glu asn val leu lys val his asp glu phe pro cys gln gln
ser ala glu asn cys asp lys ser leu his thr leu phe gly asp lys leu cys thr val ala thr leu arg glu thr
DOMAIN 1
glu gln lys ala cys cys asp ala met glu gly lyr
pro glu arg asn glu cys phe leu
val asp val glu pro arg val leu arg pro leu asn pro asn asp asp lys his gln
met cys thr ala phe his asp asn glu glu thr phe leu lys lys tyr leu tyr glu ile ala arg arg his
ala ala gln cys cys glu thr phe ala ala lys tyr arg lys ala phe phe leu leu glu pro ala tyr phe tyr pro
asp lys ala ala cys leu leu pro lys leu asp glu leu arg asp
leu arg gln lys ala ser ser ala lys gly glu
lys
1-2-3
2
3
4
5
6
TO FIG. 11B
TO FIG. 11B

FIG. 11A

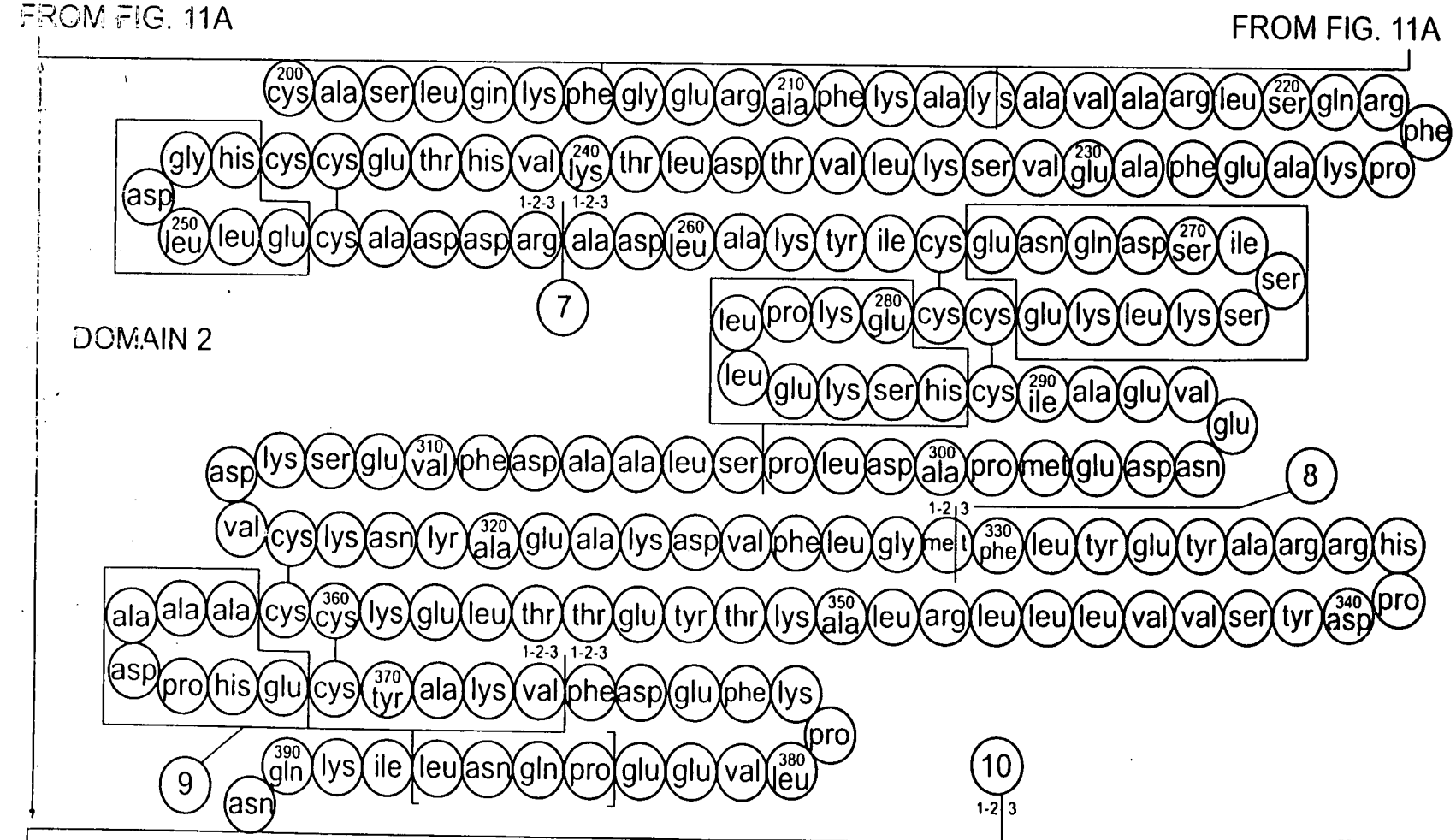


FIG. 11B

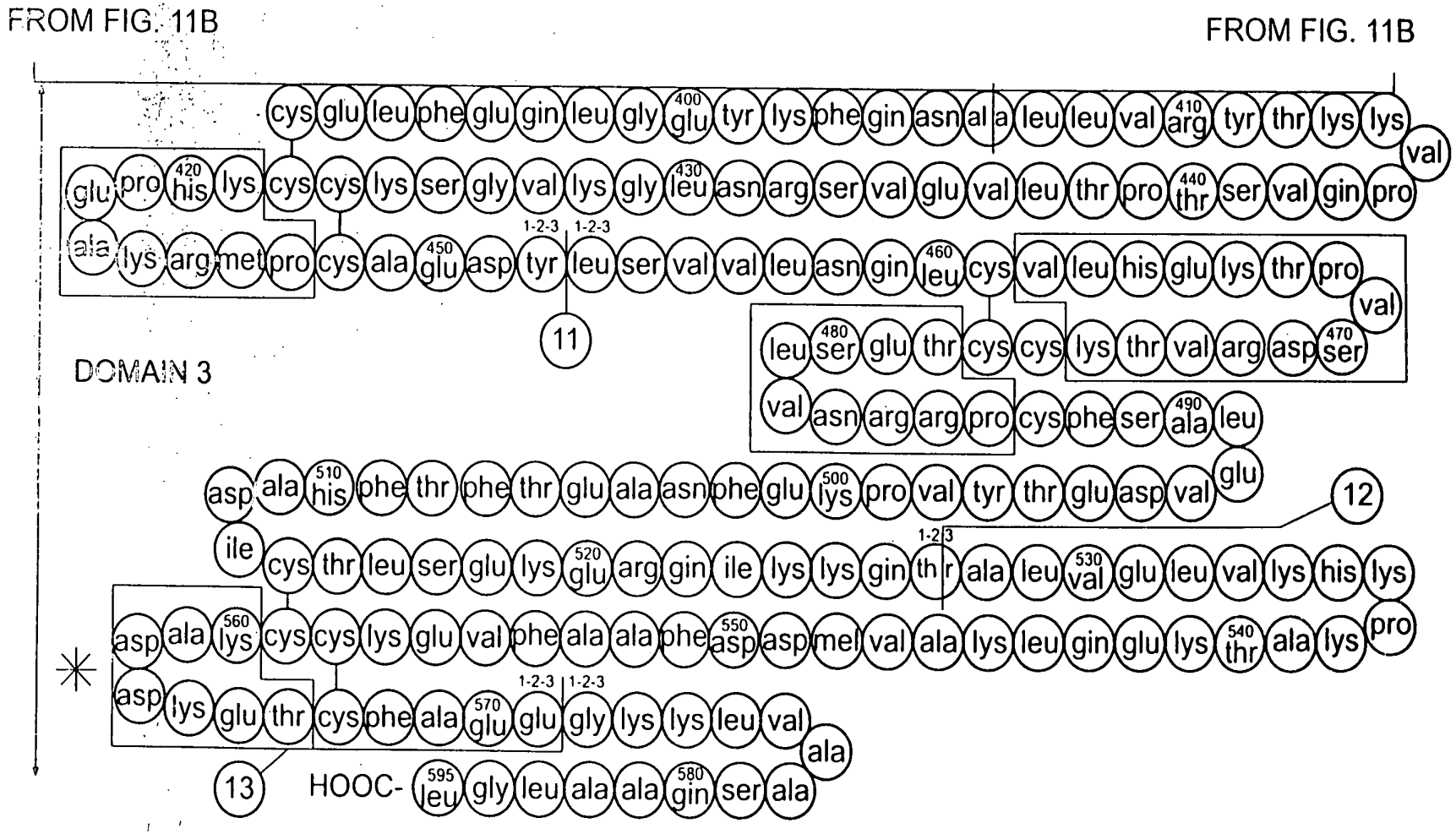


FIG. 11C

DISULFIDE BONDS SHOWN IN YELLOW

FIG. 12:
LOOP IV GLU170-A176

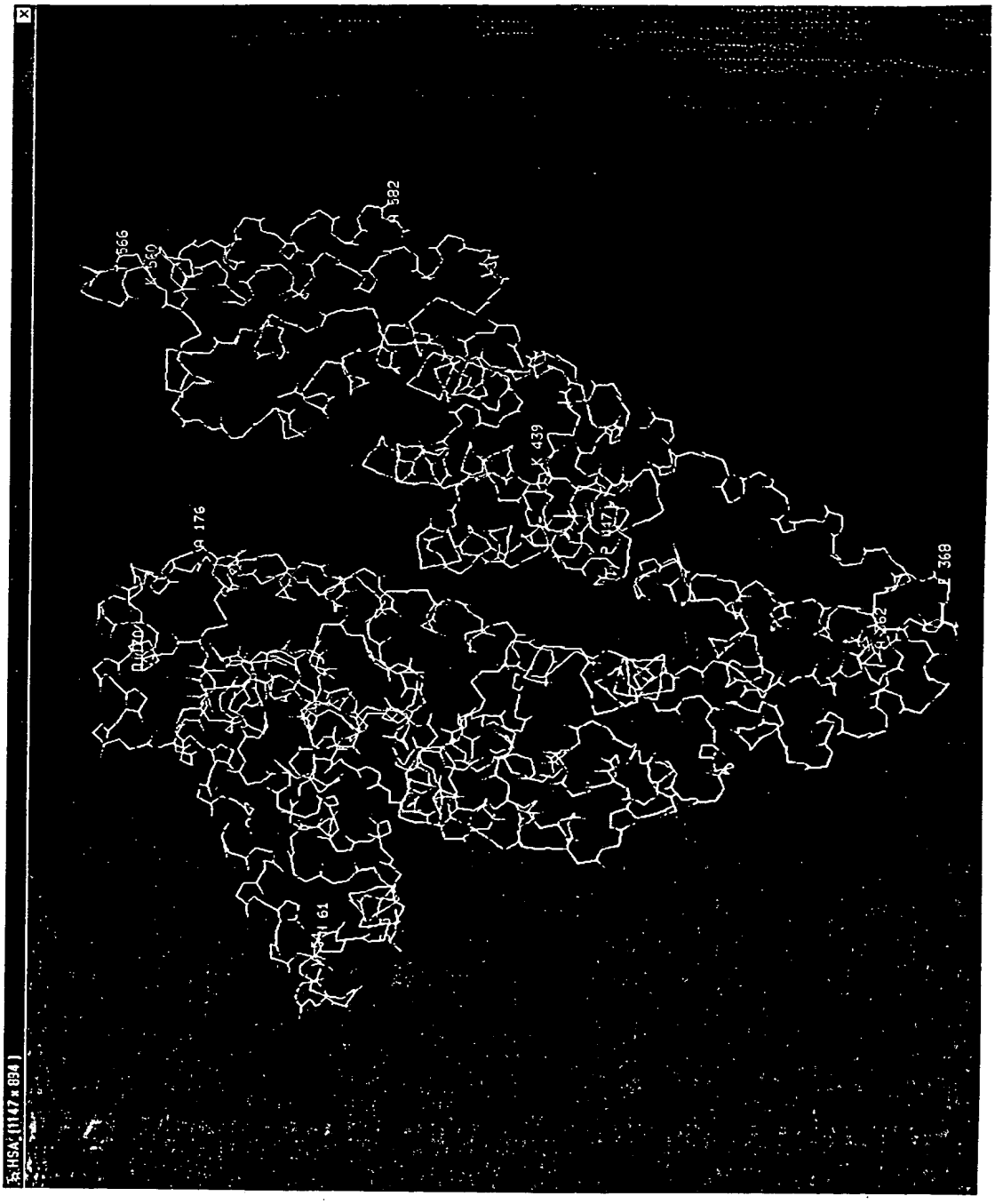


FIG. 13
TERTIARY STRUCTURE OF HA

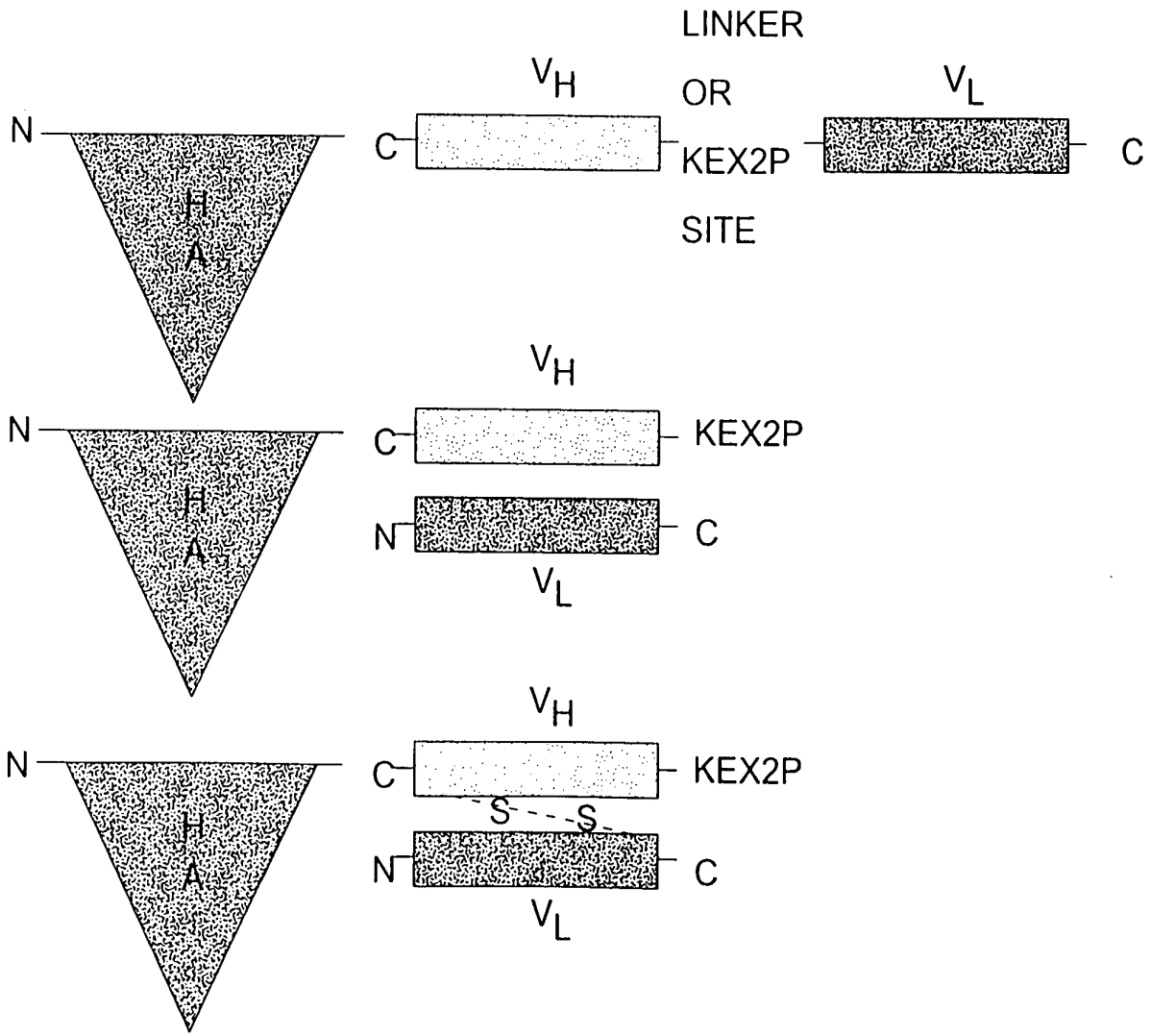


FIG. 14

```
  1 GAT GCA CAC AAG AGT GAG GTT GCT CAT CGG TTT AAA GAT TTG GGA GAA GAA AAT TTC AAA 60
  1 D   A   H   K   S   E   V   A   H   R   F   K   D   L   G   E   E   N   F   K   20

 61 GCC TTG GTG TTG ATT GCC TTT GCT CAG TAT CTT CAG CAG TGT CCA TTT GAA GAT CAT GTA 120
 21 A   L   V   L   I   A   F   A   Q   Y   L   Q   Q   C   P   F   E   D   H   V   40

121 AAA TTA GTG AAT GAA GTA ACT GAA TTT GCA AAA ACA TGT GTT GCT GAT GAG TCA GCT GAA 180
 41 K   L   V   N   E   V   T   E   F   A   K   T   C   V   A   D   E   S   A   E   60

181 AAT TGT GAC AAA TCA CTT CAT ACC CTT TTT GGA GAC AAA TTA TGC ACA GTT GCA ACT CTT 240
 61 N   C   D   K   S   L   H   T   L   F   G   D   K   L   C   T   V   A   T   L   80

241 CGT GAA ACC TAT GGT GAA ATG GCT GAC TGC TGT GCA AAA CAA GAA CCT GAG AGA AAT GAA 300
 81 R   E   T   Y   G   E   M   A   D   C   C   A   K   Q   E   P   E   R   N   E   100

301 TGC TTC TTG CAA CAC AAA GAT GAC AAC CCA AAC CTC CCC CGA TTG GTG AGA CCA GAG GTT 360
101 C   F   L   Q   H   K   D   D   N   P   N   L   P   R   L   V   R   P   E   V   120

361 GAT GTG ATG TGC ACT GCT TTT CAT GAC AAT GAA GAG ACA TTT TTG AAA AAA TAC TTA TAT 420
121 D   V   M   C   T   A   F   H   D   N   E   E   T   F   L   K   K   Y   L   Y   140

421 GAA ATT GCC AGA AGA CAT CCT TAC TTT TAT GCC CCG GAA CTC CTT TTC TTT GCT AAA AGG 480
141 E   I   A   R   R   H   P   Y   F   Y   A   P   E   L   L   F   F   A   K   R   160
```

Figure 15A

```
481 TAT AAA GCT GCT TTT ACA GAA TGT TGC CAA GCT GCT GAT AAA GCT GCC TGC CTG TTG CCA 540
161 Y   K   A   A   F   T   E   C   C   Q   A   A   D   K   A   A   C   L   L   P   180

541 AAG CTC GAT GAA CTT CGG GAT GAA GGG AAG GCT TCG TCT GCC AAA CAG AGA CTC AAA TGT 600
181 K   L   D   E   L   R   D   E   G   K   A   S   S   A   K   Q   R   L   K   C   200

601 GCC AGT CTC CAA AAA TTT GGA GAA AGA GCT TTC AAA GCA TGG GCA GTG GCT CGC CTG AGC 660
201 A   S   L   Q   K   F   G   E   R   A   F   K   A   W   A   V   A   R   L   S   220

661 CAG AGA TTT CCC AAA GCT GAG TTT GCA GAA GTT TCC AAG TTA GTG ACA GAT CTT ACC AAA 720
221 Q   R   F   P   K   A   E   F   A   E   V   S   K   L   V   T   D   L   T   K   240

721 GTC CAC ACG GAA TGC TGC CAT GGA GAT CTG CTT GAA TGT GCT GAT GAC AGG GCG GAC CTT 780
241 V   H   T   E   C   C   H   G   D   L   L   E   C   A   D   D   R   A   D   L   260

781 GCC AAG TAT ATC TGT GAA AAT CAG GAT TCG ATC TCC AGT AAA CTG AAG GAA TGC TGT GAA 840
261 A   K   Y   I   C   E   N   Q   D   S   I   S   S   K   L   K   E   C   C   E   280

841 AAA CCT CTG TTG GAA AAA TCC CAC TGC ATT GCC GAA GTG GAA AAT GAT GAG ATG CCT GCT 900
281 K   P   L   L   E   K   S   H   C   I   A   E   V   E   N   D   E   M   P   A   300

901 GAC TTG CCT TCA TTA GCT GCT GAT TTT GTT GAA AGT AAG GAT GTT TGC AAA AAC TAT GCT 960
301 D   L   P   S   L   A   A   D   F   V   E   S   K   D   V   C   K   N   Y   A   320
```

Figure 15B

```
 961 GAG GCA AAG GAT GTC TTC CTG GGC ATG TTT TTG TAT GAA TAT GCA AGA AGG CAT CCT GAT 1020
 321 E   A   K   D   V   F   L   G   M   F   L   Y   E   Y   A   R   R   H   P   D   340

1021 TAC TCT GTC GTG CTG CTG CTG AGA CTT GCC AAG ACA TAT GAA ACC ACT CTA GAG AAG TGC 1080
 341 Y   S   V   V   L   L   L   R   L   A   K   T   Y   E   T   T   L   E   K   C   360

1081 TGT GCC GCT GCA GAT CCT CAT GAA TGC TAT GCC AAA GTG TTC GAT GAA TTT AAA CCT CTT 1140
 361 C   A   A   A   D   P   H   E   C   Y   A   K   V   F   D   E   F   K   P   L   380

1141 GTG GAA GAG CCT CAG AAT TTA ATC AAA CAA AAC TGT GAG CTT TTT GAG CAG CTT GGA GAG 1200
 381 V   E   E   P   Q   N   L   I   K   Q   N   C   E   L   F   E   Q   L   G   E   400

1201 TAC AAA TTC CAG AAT GCG CTA TTA GTT CGT TAC ACC AAG AAA GTA CCC CAA GTG TCA ACT 1260
 401 Y   K   F   Q   N   A   L   L   V   R   Y   T   K   K   V   P   Q   V   S   T   420

1261 CCA ACT CTT GTA GAG GTC TCA AGA AAC CTA GGA AAA GTG GGC AGC AAA TGT TGT AAA CAT 1320
 421 P   T   L   V   E   V   S   R   N   L   G   K   V   G   S   K   C   C   K   H   440

1321 CCT GAA GCA AAA AGA ATG CCC TGT GCA GAA GAC TAT CTA TCC GTG GTC CTG AAC CAG TTA 1380
 441 P   E   A   K   R   M   P   C   A   E   D   Y   L   S   V   V   L   N   Q   L   460

1381 TGT GTG TTG CAT GAG AAA ACG CCA GTA AGT GAC AGA GTC ACA AAA TGC TGC ACA GAG TCC 1440
 461 C   V   L   H   E   K   T   P   V   S   D   R   V   T   K   C   C   T   E   S   480
```

Figure 15C

```
1441 TTG GTG AAC AGG CGA CCA TGC TTT TCA GCT CTG GAA GTC GAT GAA ACA TAC GTT CCC AAA 1500
 481 L   V   N   R   R   P   C   F   S   A   L   E   V   D   E   T   Y   V   P   K   500

1501 GAG TTT AAT GCT GAA ACA TTC ACC TTC CAT GCA GAT ATA TGC ACA CTT TCT GAG AAG GAG 1560
 501 E   F   N   A   E   T   F   T   F   H   A   D   I   C   T   L   S   E   K   E   520

1561 AGA CAA ATC AAG AAA CAA ACT GCA CTT GTT GAG CTT GTG AAA CAC AAG CCC AAG GCA ACA 1620
 521 R   Q   I   K   K   Q   T   A   L   V   E   L   V   K   H   K   P   K   A   T   540

1621 AAA GAG CAA CTG AAA GCT GTT ATG GAT GAT TTC GCA GCT TTT GTA GAG AAG TGC TGC AAG 1680
 541 K   E   Q   L   K   A   V   M   D   D   F   A   A   F   V   E   K   C   C   K   560

1681 GCT GAC GAT AAG GAG ACC TGC TTT GCC GAG GAG GGT AAA AAA CTT GTT GCT GCA AGT CAA 1740
 561 A   D   D   K   E   T   C   F   A   E   E   G   K   K   L   V   A   A   S   Q   580

1741 GCT GCC TTA GGC TTA TAA CAT CTA CAT TTA AAA GCA TCT CAG 1782
 581 A   A   L   G   L   *                                   585
```

Figure 15D